as alignment markers and the gels were exposed to Kodak BioMax™ autoradiography film. An exemplary gel is shown in Figure 1.

[0141] Bands that appeared to be possible markers for phase specific gene expression were marked on the film and aligned over the gel. The bands were excised by cutting through the film. The gel pieces were scraped from the gel and transferred to tubes and re-amplified using the same primer pairs and PCR conditions as used for incorporation of radiolabeled nucleotides.

Cloning of DNA Fragments from Differential Display

[0142] The PCR products from the gel fragments were purified, polished, ligated and cloned into XL 10-Gold Kan ultracompetent cells by heat shock with the Stratagene pCR-Script Amp SK(+) Supercompetent Cell Cloning Kit according to manufacturer's instructions. The transformed cells were spread on LB agar plates containing ampicillin, IPTG, and X-Gal each at 50 µg/ml. The plates were incubated overnight at 37°C. Plasmids containing PCR inserts were identified using blue-white colony screening. The presence of inserts was confirmed by digesting the clones with restriction endonucleases, Msc I and Nla III, followed by standard DNA gel electrophoresis. Transformants representing early, middle, and late phase embryos were sequenced using standard dideoxy protocols known in the art with the T3 primer.

Sequence Analysis

[0143] All sequences were analyzed using a program-database pair search of the NCBI BLAST 2.0 server, blastn-nr, blastn-others ests, and blastx-nr. In each case, the query sequence was filtered for low complexity regions by default and entered in FASTA format. Other formatting options were set by default; alignment view-pairwise,

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descriptions-100, and alignments-50. Using these parameter settings, significant similarity to known DNA, RNA, or protein sequences was found for several of the nucleic acid molecules of SEQ ID NOS: 1-334, for example, those described herein. (Alignment data not shown).

EXAMPLE 2: Characterization of Full Length LP2-3 cDNA Sequence

[0144] SEQ ID NO: 327, designated LP2-3, was first identified through differential display with $T_{12}MG$ and AP_1 primers (GeneHunter). The differential display band appeared to be present only in liquid suspension cultures of Loblolly Pine somatic embryos. The conditions for mRNA isolation, reverse-transcription, differential display-PCR, and gel separation/visualization for producing this band were all as described in Example 1. Likewise, the band containing the original LP2-3 fragment was excised from the differential display gel, amplified, and cloned into pCR-Script AMP SK(+) according to standard protocols known in the art.

Northern Hybridizations Demonstrating Early-Specific Expression

[0145] Northern analysis demonstrated that the LP2-3 differential display clone hybridized to an approximately 1.2 Kb mRNA from liquid suspension culture embryos but was undetectable in late (6-9) stage embryo RNA. (Figure 11) In general, LP2-3 is most highly expressed in early stage embryos in liquid culture. LP2-3 mRNA is found most abundantly in early stage somatic embryos, especially for embryos grown in liquid multiplication medium. (Figure 12) Further, transcription decreases rapidly as embryos are transferred to maturation medium (stage 3 and stage 4) and begin to mature. LP2-3 transcripts are virtually undetectable at stage 6-9 somatic embryos grown on maturation medium. (See Figure 12) Additional studies indicate that LP2-3 mRNA is expressed

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zygotically, particularly in early stage zygotic embryos, but is undetectable in mature vegetative tissues. (Figures 13 and 14) Specifically, the signal intensity from liquid suspension somatic embryo RNA was about 3 times greater than the signal from the analogous stage 1 zygotic embryo RNA. (Figures 13 and 14) LP2-3 transcripts were not detectable in total RNA from needles, stems, or roots of one year old seedlings, including those exposed to cold, ozone, wound stresses, or the hormone jasmonic acid (not shown).

LP2-3 Differential Display and 'Full-Length' cDNA Sequences

[0146] A 'full-length' cDNA was captured from SMART™ cDNA made from somatic embryo liquid suspension by using a biotinylated LP2-3 differential display fragment as a capture probe. The "full-length" cDNA was cloned and sequenced according to standard protocols known in the art. This sequence was designated at LP2-3^{+}.

[0147] GenBank blastx searches conducted with the above sequence translated in all 6 reading frames indicated that LP2-3^{+} likely encodes a member of the major intrinsic protein family. This family of proteins encodes membrane channels for the transport of water and/or ions across cell membranes. They may play a significant role in osmoregulation and may play a role in the cellular responses to water and salt stresses. As is known in the art, the MIPs are induced by dessication, flooding, and high levels of the plant hormone ABA. In contrast, the LP2-3 sequence was not detected in desiccated late-stage embryos which have high levels of ABA and, thus, appears to be regulated by some embryo-specific signal.

EXAMPLE 3: Hypothesis Development for Improved Protocols

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